ROUNDUP360

Research

PRP and chondrogenic differentiation

Please forgive *360* if we appear confused as the toing and froing that surrounds platelet-rich plasma (PRP) at the moment is truly perplexing. However, it is good when the laboratory researchers become involved, as shown by a study from **Berlin (Germany)**. Their aim was to investigate the effect of human PRP on the migration and chondrogenic differentiation of human subchondral progenitors. Human progenitors were derived from subchondral corticocancellous bone, and were analysed for their migration capacity upon PRP treatment in 96-well chemotaxis assays and cultured in high-density pellet cultures under serum-free conditions in the presence of 5% PRP. Chemotaxis assays showed that 0.1% to 100% PRP significantly stimulated the migration of corticocancellous bone compared with untreated controls. Histological staining of proteoglycan and immunostaining of type II collagen indicated that progenitors stimulated with PRP show significantly increased cartilage matrix formation compared with untreated progenitors. Real-time gene expression analysis of typical chondrocyte marker genes as well as osteogenic and adipogenic markers such as osteocalcin and fatty acid binding protein showed that PRP induces the chondrogenic differentiation sequence of human progenitors in high-density pellet cultures, while osteogenic or adipogenic differentiation was not evident. These results suggest that human PRP may enhance the migration and stimulate the chondrogenic differentiation of human subchondral progenitor cells.[1] So in the laboratory, this PRP miracle juice clearly has a positive effect.

Basic fibroblast growth factor – an indicator of degenerative disease

To monitor the severity of osteoarthritis by means other than asking a patient has many attractions, even in clinical practice. So research coming out of **Bangkok (Thailand)** strikes *360* as being of real value. The authors' aim was to investigate plasma and synovial fluid basic fibroblast growth factor (bFGF) levels in patients with primary osteoarthritis (OA) of the knee and to look at the correlation between bFGF levels and disease severity. They studied 35 patients with OA knee and 15 healthy individuals. The grading of the OA knee was performed according to the Kellgren-Lawrence classification. bFGF concentrations in both plasma and synovial fluid were determined using enzyme-linked immunosorbent assay. The researchers found that the bFGF levels in plasma and synovial fluid in patients with OA knee were significantly higher than in controls. Moreover, plasma and synovial fluid bFGF concentrations correlated positively with radiological severity. Further analysis revealed that there was a positive correlation between plasma and synovial fluid bFGF levels.[2] This, *360* feels, is interesting as the authors' findings suggest that bFGF levels may be a monitor of disease severity and could play an essential part in the pathophysiology of the degenerative process in OA.

Glucosamine works... in guinea pigs

It seems the majority of patients in *360*'s clinics are downing glucosamine by the ton. After all, OA is such a common disease that it is to be expected patients will seek assistance from health supplements. Glucosamine sulphate has been administered to patients since the early 1980s in Europe. Consequently, work from **Tokyo (Japan)** caught *360*'s eye. Histological and molecular changes were examined in order to investigate the effects of the long-term administration of glucosamine and chondroitin sulphate in a model of spontaneous OA in Hartley guinea pigs. Researchers took three groups of female three-week-old Hartley guinea pigs, the animals receiving: 1) glucosamine, 2) chondroitin sulphate and 3) neither agent, respectively. In each group, five animals were killed at eight, 12, and 18 months of age. At eight months of age, and without treatment, Hartley guinea pigs had severe degeneration of knee joint cartilage, chondrocyte apoptosis, marked reduction of tissue total RNA, decreases of aggrecan and collagen type 2 mRNAs, and increases in MMP-3 and MMP-8 mRNAs. However, long-term administration of glucosamine and chondroitin sulphate reduced cartilage degeneration at eight months of age. The marked loss of total RNA and the increase in MMP-3 mRNA were also inhibited by glucosamine and chondroitin sulphate. It thus appears that the long-term oral administration of either of these agents inhibits the progression of OA, maintains total RNA and down regulates MMP-3 mRNA for a spontaneous OA model in Hartley guinea pigs.[3] So how about for mankind, we ask at *360*? All the same it is good to know that our patients may not be wasting their time and money after all.

Randomised trials are not always what they seem

When is a trial not a trial? When it is randomised apparently, certainly in orthodontics. A paper from **Athens (Greece)** aimed to investigate whether studies published in orthodontic journals and titled as randomised clinical trials were truly randomised. A second objective was to explore the effect of journal type and other publication characteristics on the correct classification. The authors hand searched eight orthodontic journals for clinical trials labelled in the title as randomised from 1979 to July 2011. The data were analysed by using descriptive statistics, and univariate and multivariate examinations of statistical associations via ordinal logistic regression modelling (proportional odds model). There were 112 trials identified. Of those included, 33 (29.5%) were randomised clinical trials, 52 (46.4%) had an unclear status, and 27 (24.1%) were not randomised. In the multivariate analysis among the included journal types, the researchers established that the year of publication, number

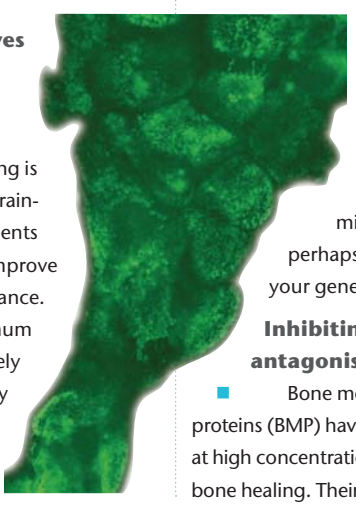
of authors, multicentre design, and involvement of a statistician were significant predictors of correctly classifying a study as a randomised clinical trial. At *360* we found this truly fascinating. Out of 112 clinical trials in the orthodontic literature labelled as randomised, only 29.5% were identified as being truly randomised based on clear descriptions of appropriate random number generation and concealment of allocation. This study indicates the need for clear and accurate reporting of clinical trials, and a requirement for educating investigators about the methodology of truly randomised clinical trials.[4] And for *360*? There is, of course, nothing randomised about us at all but we would always advocate asking a statistician to be involved in any trial, however minor the study might be.

Ossification of the ligamentum flavum – one step nearer to its cause

Ossification of the ligamentum flavum (OLF) is fairly uncommon in the West but much more evident in the Far East and commonly Japan. It will normally present with a myelopathy but its cause has been debated and remains a mystery, or so it appears to *360*. Researchers from **Fukui (Japan)**, **Hangzhou (China)** and **San Salvador (El Salvador)** have investigated the origins of this condition in more detail. Their hypothesis was that β-catenin played a role in the OLF cells in response to cyclical tensile strain. Several studies have investigated the roles of biomechanical and metabolic factors in the development and progression of OLF, based on the importance of genetic and biological factors. The process of ossification includes enchondral ossification, although such pathology remains poorly defined. The authors undertook histological, immunohistochemical, and real-time reverse transcription-polymerase chain reaction analyses of the expression of cell signalling and transcriptional factors in human OLF. Using real-time reverse transcription-polymerase chain reaction, they analysed the mRNA expression levels of signalling factors known to be involved in the ossification process (β-catenin, Runx2, Sox9, and osteopontin) in cultured OLF cells subjected to cyclical tensile strain. A Flexercell FX-3000 applied for 0, 6, 12, or 24 hours, produced cyclical tensile strain. The localisation of the signalling factors was examined in decalcified paraffin OLF sections by immunohistochemistry. Controlled samples were harvested from the nonossified ligamentum flavum of patients who underwent thoracic posterior surgical procedures. The authors found that under resting conditions (no tensile strain), the mRNA levels of β-catenin, Runx2, Sox9, and osteopontin in cultured OLF cells were significantly higher than in the control non-OLF cells. Application of cyclical tensile strain to OLF cells resulted in significant increases in mRNA expression levels of β-catenin, Runx2, Sox9, and osteopontin at 24 hours. Hypertrophic chondrocytes present around the calcification front were immunopositive for Runx2 and osteopontin. Immunoreactivity of β-catenin and Sox9 was strongly present in premature chondrocytes in the fibrocartilage area. These results suggest that cyclical tensile strain applied to OLF cells activated their ossification through a process mediated by the β-catenin signalling pathway.[5] One step nearer, it appears, to understanding the origins of this very mysterious disease.

Treadmill running improves maximum contraction force

Treadmill running is a commonly used training method for patients with spasticity to improve functional performance. Meanwhile, botulinum toxin has been widely used therapeutically in order to reduce the contraction force of spastic muscle. However, the effects of treadmill running on neuromuscular junction expression and motor unit physiology after botulinum toxin injection are not well established. To assess the effects of treadmill running on neuromuscular recovery of gastrocnemius after botulinum toxin A (BoNT-A) injection, researchers from **Taipei (Taiwan)** observed changes in gene expression. They hypothesised that the expression of acetylcholine receptor (AChR), myogenesis, and nerve plasticity could be enhanced. Consequently, 24 Sprague-Dawley rats received botulinum toxin injection in the right gastrocnemius and were then randomly assigned into untrained control and treadmill running groups. The rats assigned to the treadmill running group were trained on a treadmill three times each week with a running speed of 15 m/min for eight weeks. The duration of training was 20 mins/session. Muscle strength and gene expression of AChR subunit (α, β, δ, γ, and ε), MyoD, Myf-5, MRF_4, myogenin, p21, IGF-1, GAP_{43}, were analysed. Treadmill running had no influence on gastrocnemius mass, but improved the maximal contraction force of the gastrocnemius in the treadmill running group. Upregulation of GAP-43, IGF-1, Myo-D, Myf-5, myogenin, and AChR subunits α and β were found after treadmill running. The expression of genes associated with neurite and AChR regeneration after treadmill exercise was upregulated, which may have contributed to enhanced recovery of gastrocnemius strength.[6] So perhaps fitness is partly in your genes after all?



Inhibiting BMP antagonists

Bone morphogenetic proteins (BMP) have to be applied at high concentrations to stimulate bone healing. Their limited therapeutic efficacy may be because of the local presence of BMP antagonists such as Noggin. Noggin is sometimes known as NOG and is a protein that in humans is encoded by the NOG gene. However, because of this property, inhibiting BMP antagonists is attractive therapeutically and has been investigated in detail by a group from **Bern (Switzerland)**. The researchers hypothesised that the engineered BMP2 variant L51P stimulates osteoinduction by antagonising Noggin-mediated inhibition of BMP2. Primary murine osteoblasts (OB) were thus treated with L51P, BMP2, and Noggin. OB proliferation and differentiation were quantified with XTT and alkaline phosphatase (ALP) assays. BMP receptor-dependent intracellular signalling in OB was evaluated with Smad and p38 MAPK phosphorylation assays. BMP2, Noggin, BMP receptor Ia/Ib/II, osteocalcin, and ALP mRNA expression were analysed with real-time PCR. L51P stimulated OB differentiation by blocking Noggin-mediated inhibition of BMP2. L51P did not induce OB differentiation directly, and did not activate BMP receptor-dependent intracellular signalling through the Smad pathway. Treatment of OB cultures with BMP2 but not with L51P resulted in an increased expression of ALP, BMP2, and Noggin mRNA. By inhibiting the BMP antagonist Noggin, L51P enhances BMP2 activity and stimulates osteoinduction without exhibiting direct osteoinductive function. Indirect osteoinduction with L51P seems to be advantageous to osteoinduction with BMP2 as BMP2 stimulates the expression of Noggin, thereby self-limiting its own osteoinductive activity. Treatment with L51P is the first protein-based approach available to augment BMP2-induced bone regeneration through the inhibition of BMP antagonists. This strategy may help to decrease the amounts of exogenous BMPs currently required to stimulate bone healing.[7] More to follow, we suspect at *360*.

NSAIDs may not delay union after all

Non-steroidal anti-inflammatory

drugs (NSAIDs) are widely used in the management of post-operative and post-traumatic pain. However, it has been suggested by many sources that their use may have a deleterious effect on fracture healing. Researchers from **Daw Park (Australia)** have looked into this by means of a systematic review. Their search identified 316 papers as potentially relevant to the topic, and these were manually reviewed. The majority described small-scale studies that were retrospective or observational in nature, with limited control of potentially confounding variables, or presented little key information that was not also present in other studies. Although increasing evidence from animal studies suggests that cyclooxygenase-2 (COX-2) inhibition suppresses early fracture-healing, *in vivo* studies involving human subjects have not provided convincing evidence to substantiate this concern. The authors found no robust evidence to support a significant and appreciable problem created by the short-term use of NSAIDs after a fracture. The balance of evidence in the available literature appears to suggest that a short-duration NSAID regimen is a safe and effective supplement to other methods of post-fracture pain control, without a significantly increased risk of sequelae related to disrupted healing.[8] We found this to be greatly reassuring at *360*.

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